Bacterial genetics and physiology

Dr Laurence VAN MELDEREN, Professeur ULB

Dr Dukas JURENAS, Chargé de cours ULB

Localisation : IBMM, rue Prof. Jeener et Brachet, 12, B-6041-Gosselies

e-mail : Laurence.Van.Melderen@ulb.be, Dukas.Jurenas@ulb.be

The Bacterial Genetics and Physiology lab tackles the fundamental question in modern microbiology of bacterial cell death and survival. We mainly study the molecular mechanism of action of antibiotics and bacterial toxins and the corresponding survival or immunity mechanisms. We use modern microbiology tools allowing to follow the physiological changes at the single cell level. Our team develops biosensors, flow cytometry and microscopy techniques enabling to trace these events in time and space using fluorescent microscopy. We further study the precise molecular mechanisms *in vitro* in pure reconstituted reactions. We engineer and purify proteins and nucleic acids for enzymatic and for molecular interaction studies. To gain picture at an atomic level, we use structural biology techniques such as X-ray crystallography and Cryo-electron microscopy or tomography. Once the activity is demonstrated in a test tube, we go back to the organism level to confirm its physiological effect. Our laboratory is particularly interested in the evolution of antimicrobials, toxins and responses to these lethal agents. We characterize this evolution bioinformatically and we have all the necessary tools to follow it *in vivo* and in real time.

Van Melderen Team

Research projects

1. Antibiotics: mode of action, persistence and resistance

How antibiotics lead to the death of bacteria and how some 'lucky' cells within a clonal population cope with the antibiotic effects and manage to survive remain poorly characterized. The ability of a few cells to escape the action of an antibiotic is defined by the term 'persistence' which is characterized by a transient and reversible state during which persister cells are unable to grow. On the contrary, resistance enables cells to grow in the presence of the antibiotic. Understanding how bacterial cells are killed by the antibiotic and how persisters commit and exit from the persister state is of major importance. We analyze these questions using single-cell approaches combining microfluidics and fluorescence microscopy. We have in hand a library of biosensors allowing to track important cellular parameters such ATP/ADP ratio, ROS levels, stress responses, …. These biosensors are used to probe the physiology of dying and persister cells to determine the difference between these cell types. We also developed strategies for persister cells isolation by FACS and microfluidics sorting to establish the physiological landscape of persister cells before, during and after the antibiotic treatment, as compared to that of sensitive cells. We also want to determine whether or not these physiological characteristics are comparable for different classes of antibiotics. As model bacterium, we use *Escherichia coli* and *Acinetobacter baumannii*, a BL2 opportunistic pathogen responsible for a wide variety of infections.

2. Toxin-antitoxin modules

Toxin-antitoxin (TA) modules are widespread in bacterial genomes. They contain two genes encoding a toxin and its cognate antitoxin and are classified based on the nature and mode of action of the antitoxin. Expression of the toxin in the absence of the antitoxin leads to

inhibition of cell growth. We recently showed that the TisB toxin is induced upon antibiotic treatment and causes a series of downstream events that may contribute to survival. We are currently analyzing the mode of action of this toxin and how it could interfere with essential cellular processes. Toxins are very diverse and use a variety of molecular mechanisms to kill the cells that express them. We are interested in deciphering new mechanisms of toxicity. Moreover, we are interested in TA systems evolution and are using both experimental approaches and bioinformatics to better understand how these systems evolved and what are the selective pressures allowing for expansion and maintenance of these evolutionary successful modules.

Techniques that will be used during the research:

The questions above will be addressed using cellular biology approaches (single-cell analysis using fluorescence microscopy, microfluidics and fluorescent reporters; flow cytometry; FACS) and classical molecular biology techniques.

Selected publications of the Van Melderen team

1. Dumont B, Terradot L, Cascales E, **Van Melderen L**, Jurėnas D. (2024) Thioredoxin 1 moonlights as a chaperone for an interbacterial ADP-ribosyltransferase toxin. Nat Commun. 15(1):10388. doi: 10.1038/s41467-024-54892-w.
2. Cayron J*, Oms T*, Schlechtweg T, Zedek S, **Van Melderen L**. (2024) TisB protein is the single molecular determinant underlying multiple downstream effects of ofloxacin in *Escherichia coli*. Sci Adv. 10(13):eadk1577. doi: 10.1126/sciadv.adk1577.
3. Fraikin N, **Van Melderen L**. (2024) Single-cell evidence for plasmid addiction mediated by toxin-antitoxin systems. Nucleic Acids Res. 52(4):1847-1859. doi: 10.1093/nar/gkae018.
4. Rousseau CJ, Fraikin N, Zedek S, **Van Melderen L**. (2023) Are envelope stress responses essential for persistence to β-lactams in Escherichia coli? Antimicrob Agents Chemother. 67(10):e0032923. doi: 10.1128/aac.00329-23.
5. de Timary G, Rousseau CJ, **Van Melderen L**, Scheid B. (2023) Shear-enhanced sorting of ovoid and filamentous bacterial cells using pinch flow fractionation. Lab Chip. 23(4):659-670. doi: 10.1039/d2lc00969b.
6. Jurėnas D*, Fraikin N*, Goormaghtigh F, **Van Melderen L.** (2022) Biology and evolution of bacterial toxin-antitoxin systems. Nat Rev Microbiol. doi: 10.1038/s41579-021-00661-1.
7. Fraikin N*, Goormaghtigh F*, **Van Melderen** L. (2020) Type II toxin-antitoxin systems: evolution and revolutions. J Bacteriol. pii: JB.00763-19. doi: 10.1128/JB.00763-19.
8. Goormaghtigh F, **Van Melderen L.** (2019) Single-cell imaging and characterization of *Escherichia coli* persister cells to ofloxacin in exponential cultures. Sci Adv.5(6):eaav9462. doi: 10.1126/sciadv.aav9462.

Jurėnas Team

Research projects

1. Toxicity and secretion mechanisms of bacterial toxins

Bacteria have evolved multitude of molecular weapons to compete with other species. They secrete diversity of toxins through different secretion machineries. A particular mechanism of secretion involves injection of bacterial toxin directly into the prey upon the contact. Recently discovered mechanism delivers a single toxin molecule carefully

protected inside molecular cocoon. This molecular cocoon undergoes auto-cleavage upon which toxin will be released into the prey. We have now discovered that such cocooned toxins, called Rhs, are evolutionary conserved and can be secreted via different secretion pathways. Most importantly, their genes undergo frequent re-arrangements in the part coding for toxic domain. This leads to acquisition of novel toxins that can outcompete even the ancestor strain of the same species. We have so far mechanistically and structurally described several toxins that kill bacteria via unprecedented mechanisms – by modifying the central protein synthesis machinery, cell division machinery or by exhausting essential metabolites. A list of novel toxins with unprecedented mechanisms awaits molecular and structural description. Moreover, we have discovered novel classes of caged Rhs toxins that do not possess any secretion signals described to date. The main questions in this project are – how are toxins secreted or injected into the prey? What is the target of the toxin in the prey cell? What is the molecular mechanism of action of the toxin? How is the species secreting the toxin protecting itself from auto-intoxication?

2. *Chryseobacterium* – the golden death bacillus

Bacteria from the unexplored genus *Chryseobacterium* (Chryseos) secrete multitude of toxins and highly potent tissue-degrading enzymes. Virtually nothing is known about the factors defining the lifestyle and pathogenicity of any *Chryseos*, which comprise over 100 species with diverse lifestyles – from emerging highly morbid human pathogens, to promising biocontrol agents active against fungi, algae or other bacteria, as well as potential animal and plant symbionts. Intriguingly, *Chryseos* are non-motile bacteria, but they are able to rapidly and completely consume complex organic matter. The species *C. nematophagum*, also named "the golden death bacteria", was recently reported to destroy the pharynx tissues, enter the body cavity of a nematode prey, and digest it in 24 hours. Nevertheless, this species is highly specific to roundworms and does not invade higher organisms. We have discovered that the type 9 secretion system (T9SS) is required for pathogenesis. Infection of the *C. elegans* nematode using *C. nematophagum* expressing fluorescent reporter has shown that bacterium attached to the very beginning of the pharynx and is not passed by for the digestion. Instead, bacterium divides, kills and eventually consumes the cadaver. Using comparative genomics and proteomics we have identified putative adhesins, toxins and enzymes secreted via the T9SS by the *Chryseo* worm-killer species. Ongoing projects in the lab attempt to find what are the main virulence factors involved in attachment to host tissues and killing. Projects involve generation of mutant strains, test for worm killing phenotypes, biochemical and structural characterization of surface proteins and adhesive filaments, toxins and enzymes.

Finally, we have recently discovered gigantic proteinaceous structures compacted in the cells of *Chryseo*. Upon liberation and acidification of the medium these structures expand in telescopic fashion and could thus function as membrane puncturing device. An ongoing project in the lab questions their role in pathogenesis, the mechanism of their synthesis, heritage and strict regulation providing that only about 1% of the population synthesizes these structures.

Techniques that will be used during the research:

The questions above will be addressed using genetics (engineering and characterization of mutant and overexpression strains), molecular biology (protein purification, enzymatic characterization, protein interaction studies) and structural biology approaches (structural characterization by cryo-electron microscopy and tomography, X-ray crystallography).

Selected publications of the Jurėnas team

1. Dumont B, Terradot L, Cascales E, **Van Melderen L**, **Jurėnas D**. (2024) Thioredoxin 1 moonlights as a chaperone for an interbacterial ADP-ribosyltransferase toxin. Nat Commun. 15(1):10388. doi: 10.1038/s41467-024-54892-w.
2. **Jurėnas D**, Rey M, Byrne D, Chamot-Rooke J, Terradot L, Cascales E. *Salmonella* antibacterial Rhs polymorphic toxin inhibits translation through ADP-ribosylation of EF- Tu P-loop. Nucleic Acids Research. 2022; gkac1162.
3. **Jurėnas D***, Fraikin N*, Goormaghtigh F, **Van Melderen L**. Biology and evolution of bacterial toxin-antitoxin systems. Nature Reviews in Microbiology. 2022; 20(6):335- 350.
4. **Jurėnas D***, Talachia Rosa L*, Rey M, Chamot-Rooke J, Fronzes R, Cascales E. Mounting, structure and autocleavage of a type VI secretion-associated Rhs polymorphic toxin. Nature Communications. 2021; 12(1):6998.
5. **Jurėnas D**, Payelleville A, Roghanian M, Turnbull K, Givaudan A, Brillard J, Hauryliuk V, Cascales E. *Photorhabdus* antibacterial Rhs polymorphic toxin inhibits translation through ADP-ribosylation of 23S ribosomal RNA. Nucleic Acids Research. 2021; 49(14):8384-8395.
6. **Jurėnas D**, Journet L. Activity, delivery, and diversity of Type VI secretion effectors. Molecular Microbiology. 2021; 115(3):383-394.
7. **Jurėnas D**, **Van Melderen L**, Garcia-Pino A. Mechanism of regulation and neutralization of the AtaR/AtaT toxin-antitoxin system. Nature Chemical Biology. 2019; 15(3):285- 294.
8. **Jurėnas D**, Chatterjee S, Konijnenberg A, Sobott F, Droogmans L, Garcia-Pino A, **Van Melderen L**. AtaT blocks translation initiation by N-acetylation of the initiator $tRNA^{fMet}$. Nature Chemical Biology. 2017; 13(6):640-646.